ATTEMPTS AT PASSIVE TRANSFER OF ALLERGIC ECZEMATOUS SENSITIVITY IN MAN*,[1]

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For many years attempts have been made to passively transfer allergic eczematous contact-type hypersensitivity in man. The general opinion among dermatologists and immunologists appears to be that thus far these attempts in man have failed and that the reports of occasional successful transfers can be explained as due to technical errors, inadequate controls, etc. At least this was the conclusion in the most recent review of the pertinent literature in 1947 (Leider and Baer (1)).

Further work in this field appeared worthwhile on the basis of the publications of Landsteiner and Chase (2) and of H. S. Lawrence (3). The stimulating work of Landsteiner and Chase on experimental animals indicated that both the tuberculin-type and the contact-type allergic hypersensitivity could be passively transferred by injecting suspensions of white cells prepared from peritoneal exudates of sensitive donor animals into the blood circulation of non-sensitive recipient animals. The subsequent work of H. S. Lawrence (3) was a logical outgrowth of the experiments with animals of Landsteiner and Chase and led to the first successful passive transfer of tuberculin-type sensitivity in man. Lawrence injected viable white cells, from the blood of donor patients without active tuberculous disease, but whose skin was sensitive to tuberculin, intracutaneously into the skin of recipient patients who had previously been proven not to be skin sensitive to tuberculin. By this technic he was able to bring about a temporary passive local skin sensitivity and with larger amounts of leucocytes even a generalized skin sensitivity to tuberculin in the recipient subjects.

In the experiments which are to be reported here, the passive transfer technic worked out by H. S. Lawrence was utilized in attempts to passively transfer allergic eczematous contact-type hypersensitivity in man. This technic in general corresponds to that published by Lawrence, but some changes were made after personal communication with this author.

EXPERIMENTAL

Technic

Ninety to 120 cc. of whole venous blood was drawn from the donor patient who had been proved to have an allergic eczematous contact-type hypersensi-

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Presented at the Twelfth Annual Meeting of the Society for Investigative Dermatology, Atlantic City, N. J., June 7 and 8, 1951.

[1] We are grateful to Dr. H. Sherwood Lawrence for his generous advice and help; and to Miss Dorothy Furman and Miss Filippina Giordano, R.N. for their technical assistance.

tivity to a specific allergen. The blood was placed in potato tubes containing 1.0 ml. of a 100 mg. % heparin[2] solution for each 15 ml. of blood and the tubes were gently shaken. 0.8 to 1.0 ml. of bovine fibrinogen solution[3] was added for each 10 ml. of blood and each tube was then inverted twice.

Ten ml. aliquots of the blood containing heparin and fibrinogen were then transferred to conical centrifuge tubes and these were placed in a water bath at 37°C. for approximately 20–60 minutes (the proper time to terminate the water bath is when the white cells begin to settle on the sedimented red cells and when the supernatant plasma assumes a yellowish color). The supernatant plasma was then transferred by capillary pipette to specially constructed centrifuge tubes with capillary tips which are graduated to permit measurement of the quantity of white cells present. After centrifuging at 1800 r.p.m. for 22 minutes, the cell-free plasma was decanted and by means of a capillary pipette the packed leucocytes were taken off the red cell precipitate at the bottom of the tube and were then washed and resuspended in 2.0 ml. of Tyrode's[4] washing solution.

The cell suspensions from the several tubes were then pooled in one capillary-tipped centrifuge tube and were centrifuged at 1400 r.p.m. for 15 minutes. The supernatant was decanted and the washing in Tyrode's solution and centrifugation was repeated at 1400 r.p.m. for 15 minutes. The supernatant was again decanted, the volume of the packed leucocytes was read (average 0.08–0.14 ml. per 90 ml. of blood) and the leucocytes were resuspended in 0.15–0.25 ml. of sterile Tyrode's solution (not containing heparin).

With a 23 gauge needle and tuberculin syringe the white cell suspension was injected into the flexor aspect of the forearm of a recipient at a level slightly deeper than is customary in intracutaneous injections. The recipients were patients who were suffering from one of a variety of dermatoses, including eczematous dermatitis, nummular eczema, urticaria and atopic dermatitis, etc;

[2] Heparin—sterile solution (1,000 units per cc.) obtained from Connaught Med. Research Labs., Univ. of Toronto, in 10 ml. vials. 10 ml. heparin is added to 26 cc. sterile saline solution and to prevent clottinthis is used per 15 ml. of whole blood.

[3] Fibrinogen—Bovine fibrinogen (Armour), fraction 1, containing approximately 45 mg. fibrinogen per ml. Seven grams of bovine fibrinogen powder is dissolved in 150 cc. distilled water and placed in a water bath for 20 minutes at 37°C. This is sterilized by Seitz-filter (100 cc. funnel) and usually yields about 85–110 cc. fibrinogen solution which is stored in a refrigerator. The solution is cultured at weekly intervals to rule out contamination. The amount of fibrinogen which is used for each 10 cc. of blood varies with the different batches of fibrinogen. Therefore each batch has to be standardized individually as follows: 60 ml. whole blood is drawn from a normal subject and is placed in sterile potato tubes containing 1.0 ml. of a 100 mg. % heparin solution per 15 cc. of whole blood. The potato tubes are gently shaken and the contents are transferred in 10 ml. aliquots into 6 sterile centrifuge tubes containing 0.7, 0.8, 0.9, 1.0, 1.1 and 1.2 ml. of sterile fibrinogen solution respectively. The centrifuge tubes are inverted twice and are placed in a water bath at 37°C. for 20 to 60 minutes. The remaining part of the procedure consists of individual centrifugation, washing with Tyrode's solution, etc. as outlined below. That quantity of fibrinogen solution which is *just below* that which causes clotting is the one which is used for each 10 cc. of whole blood in all tests with the particular batch of fibrinogen.

[4] The pH of Tyrode's solution used was 7.3–7.8 after autoclaving. Tyrode's washing solution consists of Tyrode's solution 11 ml. and heparin solution 4 ml.

as a rule they were patch tested prior to the transfer test to make certain that they had no preexisting (active) allergic eczematous sensitivity to the allergen with which passive transfer was being attempted. Control sites were prepared simultaneously as described below.

The skin sites at which the white cell suspension were injected showed a nodular induration and erythema for a period of at least two days, but in most instances for several more days and at times even longer. A patch test with the eczematogenic allergen to which the donor was known to be sensitive was applied to the recipient's passive transfer site as soon as the erythema had subsided and even when the nodule persisted. The patch test usually was left in place for 2 days and in a few cases up to 7 days.

In every recipient a control site on the flexor aspect of the upper arm was patch tested at the same time. This site had been prepared by injecting it with Tyrode's solution *not* containing any white cells. In a few of the patients a patch test was also applied to a second control site on the flexor aspect of the other forearm which had been prepared by injecting a white cell suspension from the blood of a donor known *not* to be sensitive to the allergen involved in the particular experiment.

All the procedures which involved preparation and injection of the white cell suspensions were, of course, carried out under aseptic conditions. The total time interval between the drawing of the blood from the donor and the injection of the white cell suspension into the recipient was 3–4½ hours.

The tests to date have been carried out with white cell suspensions from 27 patients with allergic eczematous contact-type sensitivity. Three of these donors were strongly sensitive to two allergens and the recipients of their white cell suspensions were therefore patch tested with both. The allergens to which the donors were sensitive and to which passive transfer was attempted were as follows:

	Patients
Paraphenylenediamine, 2% in petrolatum	6
Nickel sulfate, 5% aqueous solution	7
Benzocaine, 5% in petrolatum	5
Turpentine, 50% in mineral oil	4
Ragweed oleoresin, 1:10 in acetone (Graham Labs.)	3
Mercury bichloride, 0.1% aqueous solution	1
Aquaphor®, as is	1
Ammoniated mercury, 10% in petrolatum	1
Surfacaine®, as is	1

RESULTS

The passive transfer tests were unequivocally negative in 21 of the 27 recipients. In 2 the reaction at the passive transfer site was questionable (1 with turpentine and 1 with nickel sulfate) and again was questionable on retesting 5 months later. However in 4 patients the reaction at the passive transfer site was positive. The details of these four cases are presented in table I.

COMMENT

Our results clearly indicate that in the large majority of tests, namely in 23 of 27, there was no evidence indicating passive transfer of eczematous sensitivity. The cases which we wish to discuss here in greater detail are those four in which the reaction at the passive transfer site was positive. For here the decisive question arises whether the positive reactions are an indication that passive transfer of eczematous sensitivity actually took place. In favor of such a hy-

TABLE I

Summary of four cases with positive reaction at passive transfer site

ACTIVELY SENSITIVE DONOR	ALLERGEN	DONOR'S PATCH TEST REACTION TO ALLERGEN	VOLUME OF PACKED LEUCOCYTES	VOLUME OF CELL SUSPENSION INJECTED	RECIPIENT OF WHITE CELL SUSPENSION	RECIPIENT'S REACTION TO ALLERGEN BEFORE TRANSFER	INTERVAL INJECTION TO PATCH TEST	INTERVAL PATCH TEST APPLICATION TO READING	PATCH TEST REACTION AT CELL INJECTED SITE	PATCH TEST REACTION AT CONTROL SITE ON UPPER ARM	PATCH TEST REACTION IN RETEST
			ml.	*ml.*			*days*	*days*			
M12160	Turpentine	4+	0.09	0.4	M3838	Not tested	12	9	Vesicular erythematous	Vesicular erythematous but weaker than at cell site	Vesicular erythematous (after 8 months)
M16263	Turpentine	4+	0.075	0.15	M18080	Negative	7	2	Papulovesicular erythematous	Papulovesicular erythematous	Bullous (after 2 months)
M4130	Ammoniated mercury	3+	0.09	0.1	M14494	Negative	7	7	Papulovesicular erythematous	Papulovesicular (but weaker than at cell site)	Slight papulovesicular erythematous (after 5 months)
M25122	Turpentine	4+	0.1	0.15	M20676	Negative	7	2	Erythema and edema	Erythema	Papulovesicular erythematous (after 2 months)

pothesis is the fact that three of these four patients had been patch tested with the allergen in question prior to the passive transfer tests and had had negative reactions (see table I). However, some doubt arises on this score, because, when patch tested after the cell transfer all four recipients were as sensitive or almost as sensitive at the control site as at the cell injected site. In the event of passive transfer one would have expected a stronger reaction at the cell injected site than at the control site. Moreover, when these four patients were retested 2 to 8 months after the original transfer tests they were still just as sensitive to the allergen as they had been at the time of the transfer experiment.

Had these patients undergone *passive* sensitization which then persisted for a

period of 2 to 8 months? In passive transfer tests of urticarial sensitivity according to the Prausnitz-Kuestner technic the passive sensitization often lasts over a period of many weeks, but it is confined to the site of injection and the site is usually exhausted after one exposure to the homologous allergen. H. S. Lawrence in his passive transfer tests of tuberculin-type sensitivity, using the technic developed by him and employed by us in our experiments, has observed localized and in some cases also generalized cutaneous sensitivity in the recipient. The sensitivity was localized when small amounts of white blood cells (about 0.05 ml.) were used and were injected in a single site on the forearm. Generalized cutaneous sensitivity could be induced by using 0.1 ml. or greater amounts of leucocytes, injected into multiple sites in the shoulder region of the recipients who would then be tested in an unprepared site on the opposite forearm. Passive sensitization persisted as long as 5 months (4) in an isolated instance[5]. Since there is no previous experience with Lawrence's technic when applied to transfer experiments of eczematous sensitivity, it is not possible to draw any definite conclusions either in favor of or against passive sensitization from the persistence of sensitivity in the four recipients in our series. Nevertheless on the basis of all the available information on passive transfer by other technics and in other types of allergic sensitivity one would be inclined to consider it unlikely that passively transferred sensitivity would have persisted up to 8 months.

Another piece of evidence which speaks against passive sensitization is the fact that although 9 different eczematogenic allergens were used in the 27 recipients, turpentine was the allergen in 3 of the 4 cases which showed positive reactions. Is it possible that the 4 subjects with positive reactions acquired an *active* rather than a passive sensitization? Perhaps the turpentine solution used by us had a particularly high sensitizing potential. In order to investigate this possibility we carried out patch tests with the identical turpentine solution in 8 women, who otherwise were not involved in the present experiments. Five of these 8 women were retested 2 or more months later with the same turpentine solution and in these five there were no significant differences in the results of the first tests and the retests. Thus there was no evidence in this very small control group that patch testing with this turpentine solution was capable of inducing active sensitization. Nevertheless we cannot rule out the occurrence of active sensitization in our four subjects with positive reactions in the transfer tests and the decision whether their positive test reactions were based on active or passive sensitization must be held in abeyance.

If one assumes that the reactions in these four subjects were due to *active* rather than passive sensitization this would mean that four of the 27 cell recipients developed sensitizations from the allergenic exposure entailed in the patch tests, although all 9 allergenic substances were applied in the concentrations which are

[5] There were two medical students with passively acquired sensitivity to tuberculin, who remained tuberculin positive for 1 year. One of them reverted then to the tuberculin negative state. In both these instances the possibility of *active* sensitization during ward exposure to patients with tuberculous disease in the year following cellular transfer cannot be excluded (4).

routinely employed for patch testing. As a matter of fact, there was a fifth cell recipient who became sensitized in the course of these tests. At first his patch tests with paraphenylenediamine were negative at the cell injected site and at 2 control sites. However, 2 weeks after application of the patch tests (i.e., 1 week after their removal and 3 weeks after cell injection) all three sites suddenly flared up in an eczematous reaction, indicating that this patient probably became actively sensitized to the paraphenylenediamine—unless one wishes to make the most unlikely assumption that the flare-ups were delayed evidence of passive sensitization.

Obviously, if in everyday patch testing with turpentine and other common allergens there was a danger of sensitizing 5 of 27 patients, i.e., one out of every 5 to 6 patients, the patch test could not be used as a routine testing procedure in clinical dermatology.

Again assuming that we are here dealing with active sensitization, it is possible that our results are based on statistically explainable "freak" occurrence. However, this unusually high incidence of active sensitizations from patch testing brings up the interesting possibility that, while we may not have passively transferred the eczematous sensitization to turpentine and to ammoniated mercury, we may have transferred in the white cell suspensions a *hitherto unknown and unsuspected factor* which increases the capacity of skin to become actively sensitized to the specific allergen to which the donor of the white cells is sensitive. There are a great number of factors which are known to influence the incidence of eczematous sensitization. Some of these pertain to structural characteristics and chemical properties of the allergenic substance (Landsteiner and Jacobs (5)). Others pertain to certain characteristics or pathologic changes in the human skin which is exposed to the allergenic substance. Among these are excessive or inadequate sweat secretion, excessive or inadequate sebaceous secretions, presence of inflammation or of devitalized tissue, lowered capacity to neutralize alkali (Sulzberger (6)). However, to our knowledge there has been hitherto no evidence suggesting the existence of a transferable factor which specifically favors active sensitization to the substance to which the donor of the factor possesses an allergic hypersensitivity.

SUMMARY

1. Passive transfer of eczematous allergic hypersensitivity was attempted by the technic of H. S. Lawrence: suspensions of viable white cells from donors, each one of which was known to have an eczematous allergic contact-type hypersensitivity to a specific allergen, were injected intracutaneously in recipients, known not to be allergic to the particular allergen. Transfers of white cell suspensions were carried out from 27 different donors. Among these were subjects with allergic hypersensitivity to paraphenylenediamine, nickel sulfate, benzocaine, turpentine, ragweed oleoresin, mercury, Aquaphor and Surfacaine. The recipients were patch tested at the site where the white cells had been injected and at least at one control site.

2. The results of the patch tests were negative in 21 recipients and questionable in two.

3. The results of the patch tests were positive in 4 recipients. Three of these had received white cells from donors allergic to turpentine and one from a donor allergic to ammoniated mercury. However in all four subjects patch tests were again positive on retesting 2 to 8 months later.

4. While these tests indicate that the attempt at passive transfer failed in the large majority of cases, the evidence at hand is inadequate to decide whether the sensitization was of the passively transferred type or whether it had been actively produced in the four recipients with positive tests. The possibility is considered that the white cells of patients with allergic eczematous hypersensitivity to a specific allergen may contain a factor which, when injected intracutaneously to non-sensitive subjects, increases their capacity to undergo *active* sensitization upon exposure to the particular eczematogenic allergen.

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